REMARKS/ARGUMENTS

Claims 12 and 15-17 are active.

Claim 15 is amended to remove the reference to cancelled Claim 13.

While the Examiner, in the Advisory Action, indicated that the amendments would be entered for an Appeal and Applicants are not filing an Appeal, the previously presented amendments are re-presented herein.

In the Advisory Action, the Examiner maintains that the sequence identified as SEQ ID NO:2 encoding chicken type-II collagen is obvious in view of the previously cited documents. Specifically, the Examiner also cites to the Genbank citation for chicken collagen type-II, α1mRNA asserting that all of the sequences of SEQ ID NO:2 are covered by the cited art. The Examiner further comments that those skilled in the art typically piece together various portions of clones using computer technology and the protein sequences to obtain the full-length gene.

Applicants respectfully disagree.

1. If the prior art enlightens the cloning of cDNA encoding chicken type II collagen, the cloning of such gene that has an importantly commercial value should have been completed. In fact, it took about 20 years' hard work to successfully clone the sequence shown in SEQ ID NO: 2, undergoing the longest time work in the field of cloning cDNA encoding type II collagen of all species, which demonstrates that a reasonable expectation of successfully cloning the cDNA encoding chicken type II collagen is not a reasonable conclusion, and the full length cDNA encoding chicken type II collagen could not have been obtained without a significant amount of inventive work.

Specifically, the studies on the cloning of full length cDNA encoding chicken type II collagen started from the late 1970's, and it had been reported in various papers, in particular

in papers published by the research group led by professor Upholt, from the middle 1980's and the early 1990's, that a part of minor fragments of cDNA encoding chicken type II collagen are obtained by cloning. These papers include the 6 papers of Vuorio, Young, Nah, Sandell1, Sandell2 and Upholt et al, which are cited by the Examiner. In more than 10 years, a full length cDNA encoding chicken type II collagen was not successfully cloned by these researchers, which in of itself is evidence that researchers in the relevant field would not have found it obvious to clone the full-length gene, because they did not do so.

In fact, during this same period of time, while the chicken type II full length cDNA was elusive, the full length cDNAs encoding type II collagen of several species were cloned, and the relevant time periods it took to clone those genes are depicted in figure 1 below:

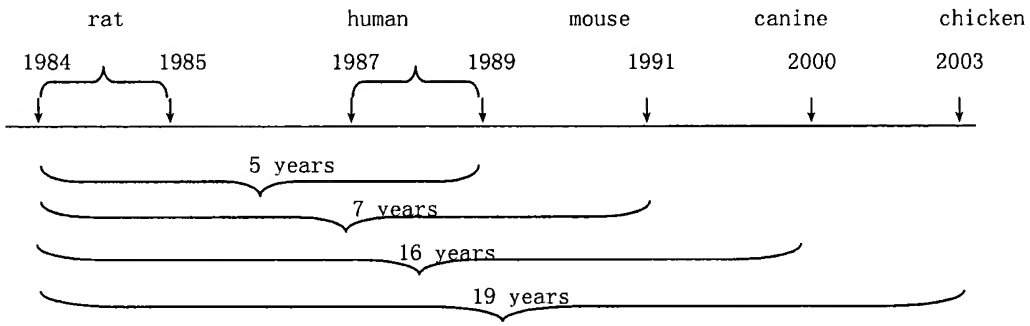

Figure 1

(1) 1984-1985: the full length cDNA encoding rat type II collagen was successfully cloned (Kohno K, et al, J. Biol. Chem., 1984, 259 (22): 13668-13673; Kohno K).

(2) 1987-1989: the full length cDNA encoding human type II collagen was successfully cloned (Elima K, et al.,Nucleic Acids Res,1987,15:9499-9504; Baldwin CT, et al., Biochem J,1989,262:521-528).

(3) 1991: the full length cDNA encoding mouse type II collagen was successfully cloned (Metsaranta M,et al.,JBC,1991, 266: 16862-16869).

(4) 2000, the full length cDNA encoding canine type II collagen was successfully cloned (Du F, et al., Gene,2000,255:307-316).

NCBI Reference Sequence: NM-204426.1 is the sequence of the present invention filed by the inventor in 2006.

Although the research group led by Prof. Upholt WB has stated that a small part of genome DNA encoding chicken type-II collagen had been cloned, only two minor fragments can be obtained.

In summary, it took about 20 years from the cloning of a gene encoding chicken type II collagen to the successful cloning of the full length cDNA encoding chicken type II collagen, which proves that it would not have been reasonably expected to obtain the full length cDNA encoding chicken type II collagen without the inventive contribution by the present inventors.

The structure of the sequence shown in SEQ ID NO: 2 is very complex, and the full length cDNA encoding chicken type II collagen cannot be obtained without the inventive contribution by the present inventors.

Prof. Upholt WB also stated in the paper published in PNAS (April 1986,: Vol.83: 2325-2329) that the full length CCOL2A1 gene sequence cannot be cloned, and pointed out that *"we have no data regarding the NH2-terminal regions of the chicken α1(II), available data for the chicken 2 (I), the human (I), the humanα1 (II), the ratα1 (II) gene and chickenα1(III) genes suggest that this region has diverged considerably."* This is intrinsic evidence that researchers (who cannot be disputed to be at least one of skill in the art) in the relevant field would not have found it obvious to clone the full-length gene, because they did not do so.

The special structural characteristics of the genomic DNA and full length cDNA encoding CCOL2A1 are additional factors that defeat any presumption that cloning the cDNA was reasonably expected with the information cited in the rejection at hand. The composition and structure of the full length cDNA are as follows:

(1) The chicken collagen type II has a very long sequence;

(2) The sequence has a high GC content;

(3) The sequence has many repeat sequences; and

(4) The sequence has many non-specifically binding sites for a primers.

What this all means is that as the genome encoding a type-II collagens is relatively big, and the GC content is high (up to 71% in the triple helical regions and in some instances up to 80%), it is a certainty that COL2A1 has a complex secondary structure, and upstream and downstream primers used to amplify and/or clone the various portions of the sequence exist in more than one location leading to non-specific binding and amplification, which was an important factor leading to the unsuccessful attempts at cloning the cDNA previously, e.g., by the Upholt group.

Further and regarding the complexities of the cDNA, the problems associated with each are discussed in more detail below:

(1) The long sequence causes problems in that it is not simple to control the selection and digestion of restriction enzymes during DNA cleavage.

(2) The high GC content is known in the art results in the formation of a stable secondary structure, hampering synthesis with the DNA polymerase on the template. Thus, there are non-specific local primer multiple renaturation sites in the template having a high GC content, resulting in non-specific amplification. Therefore, a long DNA sequence cannot be

obtained by PCR amplification from the template. As COL2A1 has a high GC content, and the GC content is up to 71% in triple helical regions and even up to 80% in some regions, it is a certainty that COL2A1 has a complex secondary structure rendering PCR amplification very difficult.

(3) The number of repeat sequences in the full length CCO2A1cDNA and genomic DNA are up to 95-98%. Because of this and after gene fragment cloning, it is difficult to distinguish the relationship of various fragments and makes it difficult to put the pieces together into a full length sequence.

(4) Non-specific binding sites result in a PCR fragments with contains a plurality of errors which are unsuitable for cloning.

These problems are the main reasons why only very short fragments (see, figure 2 attached hereto) of the full length CCOL2A1cDNA and genomic DNA were cloned previously and after extensive work and effort were the inventors able to successfully clone the full length CCOL2A1cDNA. Said another way, the cloning of the cDNA was not simply a result of piecing together known sequences as largely alleged in the rejection.

Rather, to successfully clone the full length cDNA, the inventors took a unique approach for the cloning strategy and specific primer design. More specifically,

(1) the inventors employed an effective analysis tool: the analysis of restriction enzyme sites of the sequence using Oligo 5.0 and Goldkey software so that they could address the problems of non-specific binding sites and the long sequence;

(2) the inventors designed and utilized a series of specific primers that were used to successfully amplify the CCOL2A1 full length cDNA, such as ccol2a1-1F, ccol2a1-IR, ccol2a1-2R, ccol2a1-3F, ccol2a1-3R, ccol2a1-4F, ccol2a1-4R, ccol2a1-5F, and ccol2a1-5R so that they could address the problems of number of repeat sequences and high GC content;

(3) the inventors utilized incomplete digestion with restriction enzymes and then the C-terminus was subjected to PCR amplification, so that they could address the problems of number of repeat sequences and high GC content;

(4) the inventors, to address the high GC content, used an enzyme that suitable for amplifying cDNA having a high GC content so that they could address the problems of high GC content and non-specific binding sites;

(5) the CCOL2A1cDNA was divided into 5 parts, then each part was separately subjected to PCR amplification, and each amplified fragment was made to overlap at least 50bp to link each cDNA fragment by head-to-tail using SOE-PCR so that they could address the problems of the length of the sequence and the high GC content. .

After all of this hard work, the inventors were able to successfully clone the full length cDNA. The full length cDNA comprises an open reading frame of 4260 bp and a 3'untranslated regions of 520bp (the deduced polypeptide of ccol2a1, composed of 1420 amino acids, can be divided into signal peptide, N-propeptide, N-telopeptide, triple helix, C-telopeptide and C-propeptide).

However, as PCR amplification was employed alone in the cited references, only sequences that are one fourth of the full length sequence in terms of length were obtained, but the full length cDNA was not. That is to say, according to the cited prior art, a person skilled in the art would not have reasonably expected to obtain the full length cDNA without a significant amount of work.

Following the teachings of the cited references, a person skilled in the art would not have obtained the full length cDNA.

It can be seen, form Vuorio, Nah, Sandell1 and Sandell2 led by Prof. Upholt WB and the paper of Young et al, that the research on the cloning of the cDNA and genomic DNA

encoding chicken type II collagen occurred during the time period of 1980 to 1991 and only minor fragments were cloned.

(1) It is reported in Vuorio, Nah, Sandell1 and Sandell2 led by Prof. Upholt WB that only two very short cDNA fragments, pCAR1 (525bp) and pCAR2 (680bp), are cloned (see, attached figure 2).

(2) It is reported by Young et al that only two very small cDNA fragments, pCs1 (700bp) and pCs2 (1200bp) were cloned.

As shown in the attached fig. 2, it can be seen that the four fragments are very short, only encode a small region of the 3' end of the chicken type II collagen and overlap with each other. In fact, the four cloned fragments are a single and the same gene fragment and they differ from each other only in length, where the longest one has only 1200 base pairs.

In contrast, the full length cDNA encoding chicken type II collagen obtained in the present application has a length of 4737 bp. Clearly, the cDNA encoding chicken type II collagen obtained in Vuorio, Nah, Sandell1, Sandell2 and Upholt et al comprises only one fourth of the full length cDNA encoding chicken type II collagen. Thus, the teachings of the cited references provide one fourth of the full length sequence but to obtain the remaining three-fourths, one would not have reasonably expected to obtain the full length clone without a considerable amount of effort.

A holding of obviousness cannot be sustained "unless there is some known or obvious way to make the thing or to carry out the process."[1] The CCOL2A1 gene could not have been successfully cloned without inventive skill due to the structural characteristics of the gene as

[1]See *In re Collins*, 462 F.2d 538, 174 USPQ 333 (CCPA 1972), citing *In re Hoeksema*, see *supra*.

detailed above, which was why it took more than about 20 years for the gene to be successfully cloned.

Withdrawal of the rejection is requested.

Claims 12, 15, and 16 are rejected under 35 USC 101 and under 35 USC 112, first paragraph; Claim 17 is also rejected under 35 USC 101. Specifically, in each of the rejections, the Examiner asserts that SEQ ID NO: 1 does not encode chicken type II collagen. The rejection is no longer applicable as Claim 12 (which recited SEQ ID NO:1) has been cancelled and Claim 15 has been amended to depend only from Claim 12.

Withdrawal of the rejections is requested.

Applicants submit the present application is now in condition for allowance. Early notification to this effect is earnestly solicited.

Respectfully submitted,

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